Comparative Pharmacokinetics of Perfluorobutyrate in Rats, Mice, Monkeys, and Humans and Relevance to Human Exposure via Drinking Water

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Perfluorobutyrate (PFBA) has been detected in precipitation, surface waters, water treatment effluent, and in public and private wells in Minnesota at up to low µg/l concentrations. We evaluated the pharmacokinetics of PFBA in rats, mice, monkeys, and humans to provide a rational basis for dose selection in toxicological studies and to aid in human-health-risk assessment. Studies included (1) rats—iv and oral; (2) mice—oral; (3) monkeys—iv; and (4) humans—occupationally exposed volunteers. PFBA was determined in serum (all species), liver (rats and mice), urine (rats, mice, and monkeys), and feces (rats and mice). In addition, we characterized serum PFBA concentrations in 177 individuals with potential exposure to PFBA through drinking water. Mean terminal serum PFBA elimination half-lives for males (M) and females (F), respectively, in h were (1) for rats given 30 mg/kg, 9.22 and 1.76 (oral), and 6.38 and 1.03 (iv); (2) for mice given oral doses of 10, 30, or 100 mg/kg ammonium PFBA, 13.34 and 2.87 at 10 mg/kg, 16.25 and 3.08 at 30 mg/kg; and 5.22 and 2.79 at 100 mg/kg; (3) for monkeys given 10 mg/kg iv, 40.32 and 41.04; and (4) for humans, 72.16 and 87.00 (74.63 combined). Volume of distribution estimates indicated primarily extracellular distribution. Among individuals with plausible exposure via drinking water, 96% of serum PFBA concentrations were < 2 ng/ml (maximum 6 ng/ml). These findings demonstrate that PFBA is eliminated efficiently from serum with a low potential for accumulation from repeated exposure.

Key Words: perfluorobutyrate; PFBA; pharmacokinetics; biomonitoring.

Perfluorobutyrate ($C_3F_7CO_2^-$, PFBA) is a perfluorinated alkyl carboxylate formed by industrial synthesis and by the metabolism and environmental degradation of certain fluorinated chemicals (D'Eon and Mabury, 2007; D'Eon et al., 2006; Martin et al., 2006). PFBA has recently been detected in precipitation, surface waters, and water treatment facility effluent in low ng/l concentrations (Scott et al., 2006a, b; Skutlarek et al., 2006) and in public and private wells in communities in Minnesota at up to low µg/l concentrations (http://health.state.mn.us/divs./eh/hazardous/topics/pfbasemetro.html). PFBA exposure may also result from occupational or environmental exposure to materials that can be metabolized or degraded to PFBA, such as N-alkyl-perfluorobutanesulfonamides or perfluoroalkylphonates (D'Eon and Mabury, 2007; D'Eon et al., 2006). Occupational exposure to N-alkyl perfluorobutyl ethers could also result in metabolism to PFBA via CYP450 oxidation of the ether to form the perfluorobutyryl fluoride and subsequent hydrolysis to form PFBA or the oxidation of heptafluoro-1,1-dihydrobutanol (Jay F. Schulz, personal communication).

In recent years, perfluorinated alkyl carboxylates with higher carbon numbers, such as perfluorooctanoate (PFOA), perfluoronanoate, perfluorohexanesulfonate (PFHxS), and perfluorooctanesulfonate (PFOS), have been the subject of extensive investigation due to the fact that they are found widely in blood-derived samples from the general population (Calafat et al., 2007a; Olsen et al., 2003). PFOA, PFHxS, and PFOS have low elimination rates in humans, with serum elimination half-lives of several years (Olsen et al., 2007a). Recent evidence indicates that these concentrations are declining in the United States general population (Calafat et al., 2007b; Olsen et al., 2007b), likely the result of 3M Company's decision in May of 2000 to phase out manufacturing of ammonium PFOA and materials based on perfluorooctanesulfonyl fluoride.

Unlike the eight-carbon perfluoroalkyl carboxylate, PFOA, which has been studied extensively (Kennedy et al., 2004; Kudo and Kawashima, 2003; Lau et al., 2007), there have been

no prior pharmacokinetic studies with PFBA, and information on the toxicological properties of PFBA has been limited to a few reports in the literature in which mice or rats were given PFBA in either single ip doses (Ikeda *et al.*, 1985; Kozuka *et al.*, 1991a, b; Takagi *et al.*, 1991) or in the diet for up to 2 weeks (Ikeda *et al.*, 1985; Intrasuksri and Feller, 1991; Intrasuksri *et al.*, 1998; Just *et al.*, 1989; Kozuka *et al.*, 1991a, b; Permadi *et al.*, 1992, 1993; Takagi *et al.*, 1991; Vanden Heuvel *et al.*, 1991) and in studies conducted in primary rat hepatocytes (Intrasuksri and Feller, 1991; Intrasuksri *et al.*, 1998; Vanden Heuvel *et al.*, 1991). Several of these studies evaluated hepatic biochemical and histological changes associated with peroxisome proliferation induced by perfluoroalkyl acids (Ikeda et al., 1985; Just et al., 1989; Kozuka et al., 1991a, b; Permadi et al., 1993). In general, PFBA was effective in inducing peroxisome proliferation in these rodent studies. However, the *in vivo* potency of PFBA at equivalent mass doses to PFOA was lower, particularly considering that the molecular weight of PFBA is approximately half that of PFOA (213 vs. 413 g/mol as dissociated anion). Intrasuksri and Feller (1991) studied the relative potency of a series of compounds on increasing peroxisomal fatty acid oxidation *in vitro* in primary rat hepatocytes. PFBA was found to be slightly (about 1.34 times) more potent than the cholesterol-lowering drug clofibrate. However, PFOA was 12 times more potent with respect to one enzyme (acyl-CoA oxidase) and 35 times more potent with respect to another (laurate hydroxylase). Permadi *et al.* (1992) compared the effects of several perfluorinated carboxylates on induction of xenobiotic-metabolizing enzymes and anti-oxidant enzymes in the livers of mice given the compounds in diet for 10 days. PFBA was found to increase liver cell mitochondrial and microsomal protein content as well as the activities of nicotinamide adenine dinucleotide phosphate (reduced) CYP450 oxidoreductase, superoxide dismutase, and epoxide hydrolase. Liver weight was increased and there was an apparent small increase in body weight gain. In general, at equivalent mass doses in diet, PFOA was more active and caused a significant reduction in body weight. Takagi *et al.* (1991) evaluated the ability of PFBA and PFOA to form 8-hydroxydeoxyguanosine adducts to DNA in the liver of rats after PFBA or PFOA was injected into the gut cavity of the rats at a dose of 100 mg/kg body weight. These adducts are a result of oxidative damage to DNA. PFOA increased liver weight as a percent of body weight and the amount of 8-hydroxydeoxyguanosine adducts. PFBA had no effect on either parameter. Vanden Heuvel *et al.* (1991) studied the relative potency of a series of halogenated fatty acid analogs to inhibit the enzyme, long-chain acyl-CoA synthetase, which is an essential enzyme in fatty acid metabolism. PFBA had no effect up to 2mM concentrations; whereas, PFOA produced a 50% inhibition at approximately 70–75µM concentrations.

All of the studies above, in particular the *in vitro* studies in isolated primary hepatocytes, provide evidence for reduced biological activity of PFBA against these endpoints as compared with PFOA. Ikeda *et al.* (1985) noted that the apparent reduced effect of PFBA *in vivo* as compared with PFOA may be the result of a more rapid elimination of PFBA. However, none of these studies measured tissue or blood-based matrices for PFBA, and no pharmacokinetic information was available in this or other literature.

With the finding of PFBA in public water supplies, there has been a renewed interest in the potential toxicity of this compound and its risk based on probable human exposure. Key questions concern the potential for broad species differences and sex differences within species with regard to elimination kinetics and, correspondingly, the potential for accumulation of body burden in humans upon chronic exposure. The studies reported herein were undertaken to evaluate the pharmacokinetic characteristics of PFBA in rats and mice, in order to provide a rational basis for dose selection in toxicological studies, and in monkeys and humans to aid in toxicological risk assessment in man. In addition, we report a preliminary characterization of the extent of human exposure as reflected by serum PFBA concentrations in communities potentially exposed through drinking water.

MATERIALS AND METHODS

Pharmacokinetic Studies

Materials

All chemicals used in this study were reagent-grade and were purchased from Sigma-Aldrich (St Louis, MO) or VWR (West Chester, PA). Ammonium PFBA (supplied as 28.9% solution in distilled water) and potassium PFBA (99% pure) were provided by 3M Specialty Material Division (St Paul, MN). Stable isotope $^{13}C_4$-labeled PFBA ($^{13}C_4F_7O_2H$) was supplied as the free acid in methanol (50 µg/ml, Wellington Laboratories, distributed by TerraChem, Shawnee Mission, KS) and used as internal standard for liquid chromatography/tandem mass spectrometric (LC–MS/MS) analysis of PFBA in samples from mice, rats, and humans. Perfluoropentanoate (PFPnA) was used as the internal standard for LC–MS/MS analysis of PFBA for samples from monkeys ($^{13}C_4$-labeled PFBA was not available at the time of analysis).

Laboratory Animals and Animal Care

Male and female Sprague–Dawley (SD) rats (8–10 weeks old, 200–250 g) were purchased from Charles River Laboratory (Portage, MI). Male and female CD-1 mice (8–10 weeks old, 25–35 g) were purchased from Charles River Laboratory (Raleigh, NC). Rats and mice were housed in polycarbonate, solid-bottom cages or individually in Nalgene, wire-mesh-bottom metabolism cages (Thermo Fisher Scientific, Rochester, NY) when collecting urine and feces. Harlan Teklad Diet (Madison, WI) and tap water were provided to all rats *ad libitum* throughout the study except when fasting was required, and LabDiet 5001 mouse chow (PMI Nutrition International, Brentwood, MO) and tap water were provided to all mice *ad libitum* throughout the study. Tap water was not analyzed for presence of PFBA.

Male and female cynomolgus monkeys (2.5–3 years old, 3–7 kg) were selected from an in-house colony of monkeys at Southern Research Institute (Birmingham, AL). These monkeys were purchased from Charles Rivers BRF, Inc. (Houston, TX). Certified, commercial, dry monkey chow #5048 (PMI Feeds, Inc., St Louis, MO) was fed to the monkeys two to three times each day. The diet was supplemented with fresh fruit/treats several times each week. Water was available to the monkeys *ad libitum*.

Environmental controls for the laboratory animal rooms were set to maintain a mean temperature of 20.6–23.9°C, relative humidity of 22–70%, a minimum of 10 exchanges of room air per hour, and a 12-h light/dark cycle. All studies were performed in laboratories accredited by International Association for the Accreditation of Laboratory Animal Care. All procedures involving laboratory animals were reviewed and approved by the Institutional Animal Care and Use Committee associated with the facility in which the laboratory animals were housed. Animal care and procedures followed the U.S. Department of Health and Human Services guidelines (ILAR, 1996).

Bioanalytical Procedures

All samples were analyzed by high performance LC–MS/MS methods. Analyses were completed in three bioanalytical laboratories, depending on study segment that were previously experienced in the analysis of perfluorinated carboxylates. Interlaboratory validation studies were not conducted between these three laboratories. All laboratories used Applied Biosystems-Sciex tandem mass spectrometers and software (Applied Biosystems/MDS-Sciex Instrument Corporation, Foster City, CA). Southern Research Institute used a model API 3000, 3M Medical Department Bioanalytical Laboratory (St Paul, MN) used a model API 4000, and 3M Environmental laboratory used a model API 5000. All systems were used in negative-ion mode. For PFBA analyses completed at all three laboratories, negative-ion transitions monitored were 213 atomic mass units (amu) for parent molecule to 169 amu product negative-ion. Initial work completed at Southern Research Institute (samples from monkeys) relied on ion-pairing extraction and the internal standard, PFPnA. For PFPnA, the negative-ion transition monitored was 263 amu (Bondy *et al.*, 2006) to 219 amu (product). For later analyses within 3M Company laboratories (samples from rats, mice, and humans), solid-phase extraction methods were developed using stable-isotope-labeled PFBA ($^{13}C_4$-PFBA) as an internal standard. For $^{13}C_4$-PFBA, the negative-ion transition monitored was 217 amu (Bondy *et al.*) to 172 amu (product). Internal standards were added to all extraction tubes (samples, blanks, and controls) prior to extraction. Matrix-matched standard curves were prepared by spiking PFBA into the appropriate matrix. The ranges of the standard curves were (1) 0.5–500 ng/ml of PFBA in serum or urine for samples from monkeys; (2) 2.5–100 ng/ml for samples from rats and mice; and (3) 0.5–100 ng/ml for human samples. Overall recoveries were greater than 80% and less than 120% of theoretical for all methods employed. When a sample exceeded the high standard, the sample was diluted with matrix-matched material, re-extracted, and then reanalyzed such that the quantitation was accomplished within the range of the standard curve. More specific details of methods employed for analysis of the various samples follow in the appropriate sections.

Rat Pharmacokinetic Studies

Dose dependency of absorption and elimination. Ammonium PFBA was prepared in deionized water at various concentrations ranging from 0.6 to 60 mg/ml. Male (nonfasted) and female (fasted) SD rats ($N = 3$ per sex per dose group) were given a single oral dose of ammonium PFBA at a volume of 5 ml per kg body weight so that final doses of 3–300 mg/kg were achieved. Immediately following the dosing, urine, and feces were collected from each rat for 24 h using metabolism cages. At the end of 24 h, rats were euthanized via CO_2 asphyxiation, blood (collected via abdominal aorta) and liver samples were harvested. Serum samples were obtained after blood clotting and centrifugation (2000 × g, 15 min). Serum, liver, urine, and fecal samples were stored frozen at −80°C pending analysis for PFBA.

Intravenous elimination. Male and female SD jugular-cannulated rats ($N = 3$ per sex) were given a single intravenous injection (via tail vein) of 30 mg ammonium PFBA/kg body weight. The ammonium PFBA solution was prepared in saline and 1 ml of solution was given to the rat per kg body weight. Interim blood samples (approximately ~0.5 ml) were collected from cannula at 0.25-, 0.5-, 1-, 2-, 4-, 8-, 18-, and 24-h postdose. Serum samples were obtained after blood clotting and centrifugation (2000 × g for 15 min). Serum samples were stored at −80°C pending analysis for PFBA concentration.

Oral uptake and elimination. Male and female SD jugular-cannulated rats ($N = 3$ per sex, fasted overnight) were given a single oral dose of 30 mg ammonium PFBA/kg body weight. The ammonium PFBA solution was prepared in deionized water and 5 ml of solution was given to the rat per kg body weight via oral gavage. Interim blood samples (approximately ~0.5 ml) were collected from cannula at 0.25-, 0.5-, 1-, 2-, 4-, 8-, 18-, and 24-h postdose. Serum samples were obtained after blood clotting and centrifugation (2000 × g for 15 min). Serum samples were stored at −80°C pending analysis for PFBA concentration.

Sample preparation and analysis. Analyses for samples from the studies using rats and mice (see below) were performed by the 3M Medical Department Bioanalytical Laboratory. For preparation of liver homogenates and fecal extracts, samples were allowed to thaw and approximately 0.2 g of liver or the entire collected feces were weighed and homogenized with deionized water in a clean polypropylene tube. The ratio between liver and water was 1:4 (wt/wt) and between feces and water was 1:3 (wt/wt). After the primary liver homogenization step, the whole liver homogenate was sonicated for 30 min. For feces, after the primary homogenization step, the whole homogenate was centrifuged at 2500 × g for 20 min and the corresponding supernatant was referred to as fecal extract. One hundred microliters of each serum, urine, fecal extract, or liver homogenate samples were aliquoted into clean polypropylene tubes, followed by the addition of internal standard ($^{13}C_4$-labeled PFBA). One milliliter of 1.0 N formic acid was added to all tubes, followed by 100 µl of saturated ammonium sulfate (serum only). Samples were vortexed between each addition. All extractions utilized Waters Oasis hydrophilic–lipophilic balance 3-ml columns (Waters Corporation, Milford, MA) with column conditioning, column loading, column wash, and column elution performed as described in Ehresman *et al.* (2007). A Phomenonex Synergi Polar reverse-phase column (4 µm particle size, 3.0 mm × 150 mm internal diameter) with an isocratic flow rate of 300 µl/min was used. The mobile phase was 30% acetonitrile and 70% 2mM ammonium acetate.

Pharmacokinetic analysis. Pharmacokinetic parameters were calculated from the serum concentration versus time data for PFBA using WinNonlin (Professional Version 5.1; Pharsight Corporation, Mountain View, CA). Data were subjected to one-compartmental analysis. For iv studies (rat only), a one-compartment, iv-bolus, no lag time, first-order elimination model was used. For oral uptake and elimination studies, a one-compartment, no lag time, first-order elimination model was used. Half-life ($T_{0.5}$) values were calculated from the first-order rate constant associated with the observed terminal (log-linear) portion of the serum concentration versus time curve, as estimated by linear regression.

Mouse Study

Ammonium PFBA was prepared in deionized water at 1, 3, and 30 mg/ml. Nonfasted male and female CD-1 mice ($N = 3$ per sex per dose group per time point) were given a single oral dose of ammonium PFBA at a volume of 10 ml/kg body weight so that final doses of 10, 30, and 300 mg/kg body weight were achieved. At the end of 1-, 4-, 8-, 12-, 24-, 48-, and 96-h postdose, mice were euthanized via CO_2 asphyxiation, trunk blood (collected via decapitation) and liver samples were harvested. Serum samples were obtained after blood clotting and centrifugation (2000 × g, 15 min). Urine and feces were collected for the first 24 h using metabolism cages. Serum, liver, urine, and fecal samples were stored frozen at −80°C pending analysis for PFBA concentration. Sample preparation and analysis were performed as described above for rats. Pharmacokinetic parameters were calculated as described above for rats.

Monkey Pharmacokinetic Study

A 5 mg/ml solution of potassium PFBA was prepared in sterile saline (USP, Phoenix Pharmaceutical Company, St Joseph, MO; Lot 8101069) and was stored refrigerated prior to use within 3 days of preparation. On day 0, each of the three male and three female monkeys received a single iv dose of potassium PFBA at 10 mg/kg (2 ml dosing solution/kg) into a superficial arm or leg vein. All monkeys were observed twice daily for clinical signs and were weighed on days 0, 4, 7, and 14. Urine was collected in standard metabolism cages for 24-h

intervals on the following days: prior to dose administration (day −1; baseline), on day 1 (0- to 24-h postdose), and on days 7 and 14. The volume of each urine sample was measured. Urine samples were stored frozen (approximately −20°C) prior to analysis. Blood samples (2 ml) were collected from a superficial arm or leg vein of each monkey in a restraint chair at approximately 0 (predose) minutes; 2, 4, 8, 24, and 48 h; and on days 4, 7, 11, 14, and 31 postdose. Samples were collected into tubes and were allowed to clot at room temperature. The blood samples were then centrifuged, and the serum separated and stored at −20°C until analyzed.

Monkey serum and urine analyses were completed at Southern Research Institute (Birmingham, AL). Samples (0.5 ml) were fortified with internal standard (PFPnA) followed by the addition of an ion-pairing reagent (tetrabutyl ammonium hydrogen sulfate) and extracted with ethyl acetate. The ethyl acetate layer was removed by evaporation and the resulting residue was reconstituted with mobile phase (70% 5mM ammonium acetate:30% methanol with 1.5% formic acid). After filtration with 0.2-μm syringe filter, the samples were transferred to auto sampler vials for analysis. A Keystone Scientific Aquasil C18 column (5 μm, 100 mm × 2 mm internal diameter) was used with a flow rate of 300 μl/min using a gradient elution. Gradient conditions were from 50% 5mM ammonium acetate buffer/50% formic acid (1.5% in methanol) to 25%/75%, of each mobile phase component, respectively.

Pharmacokinetic parameters were estimated from serum PFBA concentrations using WinNonlin (Version 1.2A; Scientific Consulting, Inc.; Apex, NC). The data were fit to a two-compartment model. The urinary excretion of PFBA at each collection interval was calculated and expressed as a percent of the administered dose.

Human Pharmacokinetic Studies

3M Company, Cottage Grove, Minnesota Production Worker Volunteer Study. To test elimination of PFBA in humans, a small group of 3M Cottage Grove (Cottage Grove, MN) employees who had potential occupational exposure to materials that were believed to metabolize to PFBA were asked to volunteer to participate in a postexposure, PFBA elimination study. These 3M employees were engaged in a production process that involved potential exposure to heptafluoro-1,1-dihydrobutanol as well as other related compounds, including n-perfluorobutyryl fluoride and methyl perfluorobutyrate.

After approval by the 3M Institutional Review Board, four 3M Cottage Grove employees engaged in the production process noted above from 12 to 15 May 2007 volunteered to participate in a study of the serum elimination of PFBA. A consent form was read and then signed by each study subject at the time of the first blood collection. An honorarium of $40 per each blood collection and $10 for each urine sample was provided to the employee study participants. Feasibility was established based on the initial measurement of 94.0 ng PFBA/ml serum from a sample collected on 16 May 2007 from subject no. 1. Subsequently, two of the additional three subjects who worked on this production run had initial blood collections on 22 May 2007 to determine their serum PFBA concentrations. The fourth subject was on vacation on 22 May and was not available for sampling. The results of these initial blood collections indicated the subjects could be followed to determine what the half-life of serum elimination might be for PFBA. On 30 May 2007, the subjects were voluntarily removed from the 3M Cottage Grove workplace (while receiving their normal compensation) in order to eliminate potential for any further occupational exposure to compounds that may metabolize to PFBA (e.g., heptafluorobutanol). During this time period, blood collections occurred over an eight-day period on day 0 (30 May), Day 2 (1 June), day 5 (4 June) and day 8 (7 June). Serum was immediately obtained after blood collection and frozen at −20°C. All serum samples were analyzed between 8 and 10 June 2007 with the exception of Subject no. 1. Subject no. 1 had a serum PFBA concentration above the LLOQ (0.5 ng PFBA/ml serum) on day 8 and remained voluntarily removed from any potential occupational exposure for an additional seven days, until 14 June, at which time a final blood collection was obtained and serum subsequently analyzed.

Sample preparation and analysis: Analysis of human sera for PFBA was conducted by the 3M Company Environmental Laboratory. PFBA was extracted from the human serum by protein precipitation in acetonitrile via a MultiPROBE II robotic liquid handling system. Samples were prepared in centrifuge tubes with carbon added instead of a 96-well plate format. These samples were then centrifuged at 9000 × g for 20 min. Quality control spikes and blanks were extracted in control human plasma. A Prism reverse-phase column (5 μm, 50 mm × 2 mm internal diameter) was used with a flow rate of 300 μl/min using a gradient elution. Gradient conditions were from 60% 2mM ammonium acetate containing 0.01% acetic acid buffer/40% methanol to 5%/95%, of each mobile phase component, respectively.

Pharmacokinetic analysis: For each of the three subjects in the initial time-course study, the data were examined by semilog plots which indicated that a first-order elimination analysis was applicable for this particular analysis. The elimination constant, λ, was estimated based on the first-order relationship $(C_t = C_0 \cdot e^{-\lambda t})$, and the elimination half-life $(T_{0.5})$ was determined from the relationship, $\lambda = 0.693/T_{0.5}$. Ninety-five percent confidence intervals (95% CI) were calculated for each $T_{0.5}$ that used the upper and lower limits of the 95% CI for elimination constant, λ. Graphics and statistical analyses were performed using SAS JMP (SAS Corporation, Cary, NC) and Excel (Microsoft Corporation, Bellevue, WA).

3M Company, Cordova, Illinois Production Worker Volunteer Study. Due to the limited number (3) and single sex (male) of 3M Cottage Grove plant site employees involved in the first study of human elimination of PFBA, a second study was conducted to obtain additional serum PFBA elimination measurements of other potentially exposed 3M workers in order to (1) increase the study sample size to confirm the relatively rapid serum elimination rate for PFBA; and (2) include both male and female employees. An employee biomonitoring study completed in the Fall of 2006 had shown that 28 3M Cordova (Cordova, IL) Electronic Materials factory employees had a geometric mean serum PFBA concentration of 8 ng/ml (95% CI 4.9–13.1 ng/ml) with a range of less than the lower limit of quantitation (LLOQ, 0.5 ng/ml) to 56.7 ng/ml. Potential exposure to PFBA was likely via metabolism of precursor compounds that may have included hydrofluoroethers and perfluorobutyryl fluoride.

After protocol approval by the 3M Institutional Review Board, the 3M Cordova Electronic Materials factory employees were asked to consider voluntarily participating in a study designed to collect blood samples of individuals who may be absent from the workplace (e.g., on vacation) for a minimum of 7 days from mid-August through September 2007. The assumption was that these employees would likely have measurable PFBA concentrations similar to the subset of samples used in the first study, above. Participants were required to read and sign a consent form at the time of the first blood and urine collection. An honorarium of $100 was provided to each subject who participated in this study by providing two serum and urine samples.

Seven male and three female employees volunteered. Participating employees provided a blood sample (approximately 3–5 ml) immediately prior to their leaving the plant to go on their vacation and immediately upon return from vacation prior to re-entering the plant. The minimum elapsed time was seven days. Upon returning for the second blood collection, every employee participant was asked to confirm that they had not re-entered the plant since the first blood collection. Spot urine samples were also provided at the time of the blood collection.

Upon collection of each blood sample, the sample was allowed to clot and the serum removed and then frozen at −20°C. Each sample was identified as to the employee, and day and time of collection. Upon collection of all participants' samples between mid-August and the end-of-September, the serum samples were shipped, with frozen ice bags, via overnight delivery to the 3M Medical Department. Upon receipt of the samples the next morning, each serum sample was split into two different tubes with personal identifiers removed. These split samples were then immediately taken to the 3M Environmental Laboratory (St Paul, MN) for a blinded analysis for PFBA as described above for the Cottage Grove, Minnesota Production Worker Volunteer study.

Pharmacokinetic analysis: The average of the split samples was used in the estimation of the serum elimination half-life for PFBA based on the change in concentration between the initial and return-to-work samples. For the ten

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TABLE 1
Mean ± SE Values for Serum and Liver Concentration of PFBA and Percent of Given Single Oral Dose in Urine and in Feces 24 h after Dosing in SD Rats over a Range of Ammonium PFBA Doses ($N = 3$ per Group Except as Indicated)

		Single oral dose (mg ammonium PFBA/kg body weight)				
		3	10	30	100	300
Serum [PFBA] (μg/ml)	Male	4.53 ± 0.77	15.60 ± 3.56	15.77 ± 2.91	57.30 ± 12.85	33.80 ± 11.10
	Female	NS[a]	0.01 ± 0.00	0.20 ± 0.16	0.25 ± 0.09	0.17 ± 0.05
Liver [PFBA] (μg/g)	Male	1.18 ± 0.25	3.50 ± 0.82	3.46 ± 0.82	15.55 ± 4.24	NS
	Female	NS	NS	NS	NS	NS
% Dose in urine	Male	63.78 ± 11.68	59.60 ± 6.25	62.53 ± 8.04	50.99 ± 4.35	90.16 ± 2.75
	Female	NS	112.37 ± 7.09	102.65 ± 5.24	100.68 ± 0.37[b]	107.85 ± 3.48
% Dose in feces	Male	0.10 ± 0.10	0.43 ± 0.30	2.99 ± 2.77	0.19 ± 0.10	1.25 ± 1.12
	Female	NS	0.00 ± 0.00	0.62 ± 0.32	0.16 ± 0.02[b]	0.11 ± 0.03

[a]NS = not studied.
[b]Excludes data for an individual female for which there was evidence that spillage of urine into the fecal collection cup had occurred. This female had 68% of dose found in collected urine and 30% of dose found in collected feces.

subjects in the Cordova, Illinois Production Worker Volunteer elimination study, λ was estimated for each of the nine subjects who had an initial serum concentration above the LLOQ. Calculations were based on the one-compartment, first-order relationship, as described above. Use of a one-compartment, first-order model was believed to be supportable based on the results obtained for the three 3M Cottage Grove employee volunteers, which demonstrated that a first-order model was adequate in describing the serum elimination profile of PFBA based on multiple sampling time points.

Biomonitoring Study

Preliminary Characterization of Human Serum PFBA Concentrations from Exposure to PFBA via Drinking Water

The purpose of the human biomonitoring study was to better understand the potential human exposure to PFBA via drinking water in the areas of Washington and Dakota Counties in Minnesota, a region of the Minneapolis–St Paul metropolitan area (http://health.state.mn.us/divs./eh/hazardous/topics/pfbasemetro.html). In a study design approved by the 3M Institutional Review Board, current and former 3M employees with personal mailing addresses located in communities with potential exposure to PFBA through municipal water supplies and private wells and for which stored (-70°C) serum samples existed from a Fall of 2005 through Spring of 2006 voluntary serum monitoring program for PFOS and PFOA were approached by mail and asked to allow the analysis of their stored serum sample for PFBA. Occupational exposure was the primary source of exposure to PFOS and PFOA for the serum samples obtained in 2005. Of the 201 former ($N = 136$) and current ($N = 65$) 3M Cottage Grove employees that were asked to participate in the study, a total of 179 (89.1%) returned a signed consent form and had their stored serum sample analyzed for PFBA. Analysis of PFBA in the stored sera samples was performed by LC–MS/MS as described above for the Cottage Grove, Minnesota Production Worker Volunteer Study. Two male responders were found to have had occupational exposure that occurred close to the time of sampling in 2005, which led to elevated PFBA serum concentrations, and data for these two males were excluded from the overall analysis.

Measured PFBA concentrations were related via descriptive analyses to the questionnaire responses. Analyses were stratified by former versus current employee status because the potential for occupational exposure might exist for the latter group. Data were summarized in several ways, including by location, employment status, and water source. Cumulative probabilities were determined.

RESULTS

Pharmacokinetic Studies

Dose dependency of absorption and elimination in rats. The dose dependency of serum and liver PFBA concentrations 24 h after a single oral dose and the percent of dose eliminated during 24 h after dosing are presented in Table 1 as mean ± standard error (SE). Mean liver concentrations ranged from 22 to 27% of mean serum concentrations for male rats. Liver concentrations were not measured for female rats due to the low serum concentrations observed at 24 h. Of the PFBA dose administered, approximately 51–90% and 101–112% was excreted in the urine of male and female rats, respectively, within the first 24 h, whereas the percent of dose eliminated in feces in this time period ranged from 0% (based on values below the limit of quantitation) to approximately 3%.

Intravenous elimination study in rats. Table 2 provides the mean ± SE for several pharmacokinetic parameters estimated from the iv dose elimination study in jugular-cannulated rats. Mean data by sex are represented graphically in Figure 1. After a single iv dose of 30 mg ammonium PFBA/kg, there was a substantial difference in mean serum PFBA elimination constants (λ) for males and females (0.109/h and 0.673/h, respectively) resulting in mean elimination half-lives ($T_{0.5}$) of 6.38 and 1.03 h, respectively. Mean volume of distribution (V_{dss}) estimates differed somewhat between males and females (253 and 187 ml/kg, respectively) but, in both cases, were in the range typically associated with extracellular distribution (approximately 200 ml/kg) (Wagner, 1975). The sex difference in estimated V_{dss} may have been influenced by the sex difference in λ.

Oral uptake and elimination study in rats. Table 2 also provides the mean ± SE for several pharmacokinetic parameters estimated from the oral uptake and elimination

TABLE 2
Mean ± SE Values for Pharmacokinetic Parameters in SD Rats Given either a Single Oral or a Single *iv* Dose of 30 mg Ammonium PFBA/kg Body Weight ($N = 3$ per Group)

Parameter	Sex	Oral	iv
K_a (1/h)	Male	3.04 ± 0.43	N/A[a]
	Female	4.15 ± 2.35	N/A
T_{max} (h)	Male	1.25 ± 0.12	N/A
	Female	0.63 ± 0.23	N/A
C_{max} (µg/ml)	Male	131 ± 5	118 ± 3
	Female	136 ± 12	161 ± 2
Serum [PFBA] at 24 h (µg/ml)	Male	28.68 ± 12.67	3.54 ± 1.16
	Female	0.14 ± 0.06	0.13 ± 0.04
λ (1/h)	Male	0.075 ± 0.006	0.109 ± 0.009
	Female	0.393 ± 0.057	0.673 ± 0.019
$T_{0.5}$ (h)	Male	9.22 ± 0.75	6.38 ± 0.53
	Female	1.76 ± 0.26	1.03 ± 0.03
CL (ml/h)	Male	4.63 ± 0.28	7.98 ± 0.57
	Female	14.32 ± 1.36	27.65 ± 0.55
AUC (µg · h/ml)	Male	1911 ± 114	1090 ± 78
	Female	443 ± 42	239 ± 5
V_{dss} (ml/kg)	Male	209 ± 10	253 ± 6
	Female	173 ± 21	187 ± 3

Note. AUC, area under curve; CL, clearance.

[a]N/A = not applicable (iv-bolus dose).

study in fasted jugular-cannulated rats. Mean data by sex are represented graphically in Figure 2. After a single oral dose of 30 mg ammonium PFBA/kg, mean C_{max} serum PFBA concentrations were essentially the same in males and females; however, T_{max} values were 1.25 and 0.63 h for males and females, respectively. Absorption rate (K_a) was quite similar for males and females, with means of approximately 3/h and

4/h, respectively. As in the iv study, mean λ for serum elimination of PFBA were also quite different between males and females (0.075/h and 0.393/h, respectively), resulting in mean $T_{0.5}$ values of 9.22 and 1.76 h, respectively. Also similar to the iv study, mean V_{dss} estimates differed somewhat between males and females (209 and 173 ml/kg, respectively) but, in both cases, were also in the range typically associated with extracellular distribution and may have been influenced by the sex difference in λ.

Mouse studies. Table 3 provides the mean ± SE for several pharmacokinetic parameters estimated in male and female mice from data obtained after single oral doses of 10, 30, or 100 mg ammonium PFBA/kg. Mean data by sex are represented graphically in Figure 3 (males) and Figure 4 (females). The mean serum elimination $T_{0.5}$ values in male mice were approximately four to five times those in female mice at PFBA doses of 10 and 30 mg/kg (13.34 vs. 2.87 h at 10 mg/kg and 16.25 vs. 3.08 h at 30 mg/kg for males and females, respectively). However, at the 100 mg/kg dose, the male mean $T_{0.5}$ of 5.22 h was approximately one-third that estimated for males at 10 and 30 mg/kg; whereas, the female mean $T_{0.5}$ of 2.79 h at the 100 mg/kg dose was similar to the female values estimated for the 10 and 30 mg/kg doses. Similar to the data from rats, male mice had higher V_{dss} estimates than females at 10 and 30 mg/kg, but V_{dss} was slightly lower at 100 mg/kg. As in rats, these V_{dss} estimates were in a range consistent with predominant extracellular distribution. The faster $T_{0.5}$ for male mice at the 100 mg/kg dose suggests that the simple one-compartment model is not adequate to describe the kinetic data at 100 mg/kg and that a two-compartment model may be more appropriate. On average, the percent of dose recovered in urine of female mice after 24 h (range of means approximately 65–68%) was approximately twice that recovered in urine of male
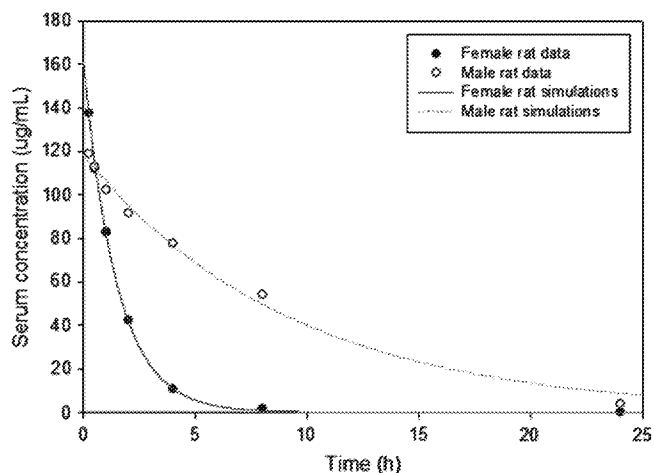

FIG. 1. Mean serum PFBA concentrations over time in male (open circles) and female (closed circles) jugular-cannulated SD rats ($N = 3$ per sex) after a single iv dose of 30 mg $NH_4^+PFBA^-$/kg body weight.
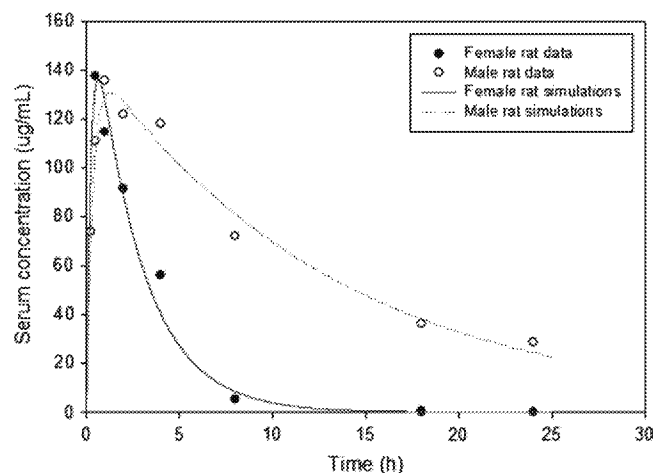

FIG. 2. Mean serum PFBA concentrations over time in male (open circles) and female (closed circles) jugular-cannulated SD rats ($N = 3$ per sex) after a single oral dose of 30 mg $NH_4^+PFBA^-$/kg body weight.

TABLE 3

Mean ± SE Values for Pharmacokinetic Parameters in CD-1 Mice Given Single Oral Doses of Ammonium PFBA ($N = 3$ per Group Except as Indicated)

Parameter	Sex	Single oral dose (mg ammonium PFBA/kg body weight)		
		10	30	100
K_a (1/h)	Male	4.28 ± 13.97	9.80 ± 4562.70	0.40 ± 0.19
	Female	0.68 ± 0.11	1.15 ± 0.20	1.17 ± 0.26
T_{max} (h)	Male	1.04 ± 2.63	0.56 ± 212.5	4.14 ± 0.57
	Female	2.35 ± 0.14	1.76 ± 0.16	1.68 ± 0.18
C_{max} (µg/ml)	Male	50.50 ± 5.81	119.46 ± 13.86	278.08 ± 20.38
	Female	52.86 ± 2.08	151.20 ± 6.92	187.97 ± 15.90
Serum [PFBA] at 24 h (µg/ml)	Male	21.6 ± 4.96	51.93 ± 12.04	121.33 ± 3.21
	Female	1.52 ± 0.36	4.13 ± 1.68	6.37 ± 3.87
Liver [PFBA] at 24 h (µg/g)	Male	4.92 ± 0.73	11.13 ± 0.79	34.47 ± 0.41
	Female	0.26 ± 0.04	0.72 ± 0.22	0.96 ± 0.27
% Dose in urine at 24 h	Male	34.58 ± 9.29	35.16 ± 4.56	34.79 ± 6.01
	Female	66.88 ± 27.28	67.98 ± 23.46[a]	65.44 ± 44.78
% Dose in feces at 24 h	Male	4.10 ± 1.83	6.87 ± 1.75	10.92 ± 2.1
	Female	6.35 ± 3.33	5.62 ± 2.49[a]	6.64 ± 2.65
$T_{0.5}$ (h)	Male	13.34 ± 4.55	16.25 ± 7.19	5.22 ± 2.27[b]
	Female	2.87 ± 0.30	3.08 ± 0.26	2.79 ± 0.30
CL (ml/h)	Male	0.35 ± 0.09	0.37 ± 0.80	0.98 ± 0.14
	Female	0.76 ± 0.03	0.87 ± 0.04	1.67 ± 0.08
AUC (µg · h/ml)	Male	1026 ± 248	2869 ± 6116	3630 ± 530
	Female	387 ± 14	999 ± 42	1760 ± 88
V_{dss} (ml/kg)	Male	152	296	207
	Female	107	134	229

Note. AUC, area under curve; CL, clearance.

[a]Excludes data for one female for which 25.74% of dose was recovered in collected feces and 17.88% was recovered in collected urine, indicating possible spillage of urine into feces container.

[b]The faster $T_{0.5}$ for male mice at the 100 mg/kg dose suggests that the simple one-compartment model is not adequate to describe the kinetic data at 100 mg/kg and that a two-compartment model may be more appropriate.

mice (all means approximately 35%). The presence of PFBA in feces of male and female mice (range of means from approximately 4–11%) suggests incomplete absorption, biliary elimination, or, more likely, some combination of both. Mean liver concentrations ranged from 18 to 43% of mean serum concentrations for male mice, increasing with dose, and 12 to 16% for female mice with no dose dependency.

Monkey intravenous dose pharmacokinetic study. Table 4 provides the mean ± SE for several pharmacokinetic parameters obtained from the monkey iv elimination study. The serum elimination half-lives for α and β phases, respectively, were 1.61 ± 0.06 h and 40.32 ± 2.36 h for males, and 2.28 ± 0.14 h and 41.04 ± 4.71 h for females. Within the first 24 h in male and female monkeys, 41 and 46% of the administered PFBA doses were excreted in urine, respectively. Figure 5 presents semilog plots of serum PFBA concentrations vs. time for the three male and three female monkeys, respectively.
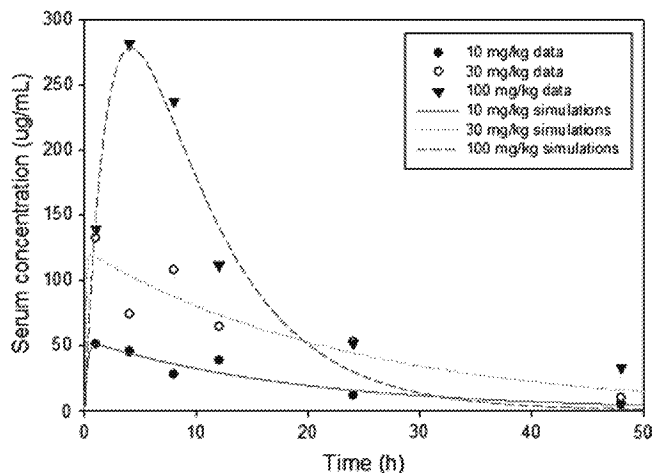

FIG. 3. Mean serum PFBA concentrations over time in male CD-1 mice ($N = 3$ per time point per dose) after a single oral dose of 10 (closed circles), 30 (open circles), or 100 (closed triangle) mg $NH_4^+PFBA^-$/kg body weight.
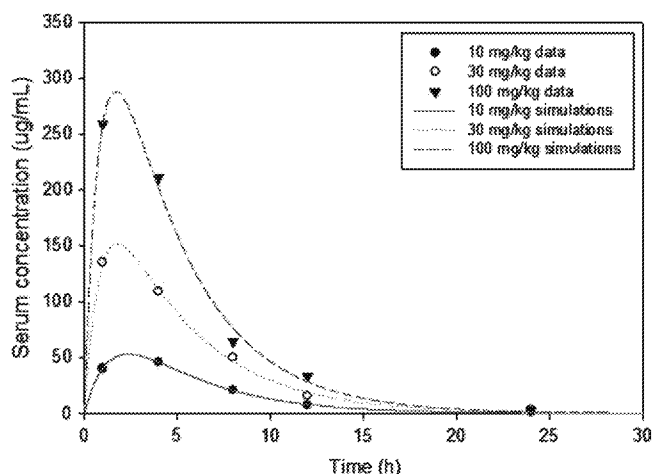
FIG. 4. Mean serum PFBA concentrations over time in female CD-1 mice ($N = 3$ per time point per dose) after a single oral dose of 10 (closed circles), 30 (open circles), or 100 (closed triangle) mg $NH_4^+PFBA^-$/kg body weight.

Human pharmacokinetic study. The results of the individual serum PFBA elimination $T_{0.5}$ calculations for 3M Cottage Grove employees are presented in Table 5. The calculations excluded the time period during which the employees may have had occupational exposure to compounds that could potentially metabolize to PFBA. In these three employees, the serum PFBA elimination $T_{0.5}$ ranged from 28.6 to 109.7 h (1.2 to 4.6 days). Figure 6 shows the corresponding semilog plots of serum PFBA concentrations vs. time for the three study subjects.

Data for the ten 3M Cordova employees are shown in Table 6. The first date of blood draw was 8 August 2007 and the last day was 25 September 2007. The minimum and maximum amount of time between samples collected were 165 and 234 h, respectively. Of the 10 participants, one subject (female) had initial split serum samples that either approached (0.8 ng/ml) or

measured at less than the LLOQ (< 0.5 ng/ml). Therefore, this participant was not included in the estimation of the serum PFBA elimination $T_{0.5}$ of PFBA. The remaining nine participants had split sample initial serum PFBA concentrations greater than the LLOQ although three subjects were measured at < LLOQ upon their return to work. To calculate λ, these < LLOQ final PFBA concentrations were analyzed using the value of the LLOQ.

Table 6 provides the initial and final serum PFBA concentrations, elapsed time that the individual was gone from the workplace, and the serum elimination half-life expressed in h and days where the subject's final serum PFBA concentration was considered to be < LLOQ. The range in serum PFBA elimination $T_{0.5}$ values for the nine participants for which this was estimated was 44–152 h (1.9–6.3 days). The arithmetic mean serum PFBA elimination $T_{0.5}$ was 72 h (95% CI 43–101 h), equivalent to 3.0 days (95% CI 1.8–4.2 days). The median serum elimination half-life was 55 h (2.3 days). There was no difference in the serum elimination half-lives reported for the two female employees compared with the seven male employees.

The serum PFBA elimination $T_{0.5}$ was also calculated assuming an imputed value when the final serum PFBA concentrations were reported at < LLOQ (i.e., $0.5/\sqrt{2} = 0.35$ ng/ml). The results did not substantially differ. With the imputed value, the arithmetic mean serum PFBA elimination $T_{0.5}$ was 68 h (95% CI 41–96 h), equivalent to 2.9 days (1.7–4.0 days).

Biomonitoring Study

Human biomonitoring results. 3M employment status at the time of sample collection (Fall of 2005 through Spring of 2006) by gender, age, and self-reported residential water source (municipal or private well) among the 177 nonoccupationally exposed participants is shown in Table 7.

Table 8 provides data on the distribution of serum PFBA concentrations. A total of 127 of 177 (71.8%) of the sera samples analyzed were reported at the LLOQ of 0.5 ng/ml (Table 8). For former employees, 73.2% of the serum samples (93 of 127) were below LLOQ compared with 68.0% (34 of 50) for current employees (Table 8). Of the 177 individual samples, 96.1% were measured at less than 2 ng/ml PFBA (Table 8, Fig. 7). One non-occupationally-exposed current employee had a serum concentration above 2.0 ng/ml which was 2.2 ng/ml. Among the former employees, the highest PFBA serum concentration was 6.2 ng/ml followed by 5.3 ng/ml, 3.1 ng/ml, and three individuals between 2.1 and 2.5 ng/ml. All other former employees had serum PFBA concentrations below 2 ng/ml. Of former employees, 76.4% (90 of 127) had municipal water compared with 72.0% (36 of 50) of current employees. Of the six individuals who had serum PFBA concentrations above 2 ng/ml, only one had a private well, and that individual's serum PFBA concentration was 6.2 ng/ml.

TABLE 4
Mean ± SE Values for Pharmacokinetic Parameters in Cynomolgus Monkeys Given a Single *iv* Dose of 10 mg Potassium PFBA/kg Body Weight

Parameter	Males	Females
Serum [PFBA] at 0.5 h (μg/ml)	35.25 ± 2.99	37.15 ± 2.29
Serum [PFBA] at 24 h (μg/ml)	0.079 ± 0.016	0.162 ± 0.027
% dose in urine at 24 h	41.69 ± 5.83	36.20 ± 7.62
$T_{0.5}\alpha$ (h)	1.61 ± 0.06	2.28 ± 0.14
$T_{0.5}\beta$ (h)	40.32 ± 2.36	41.04 ± 4.71
AUC (μg · h/ml)	112 ± 6	159 ± 8
CL (ml/h)[a]	494 ± 61	224 ± 19
V_{dss} (ml/kg)	526 ± 68	443 ± 59

Note. AUC, area under curve; CL, clearance.
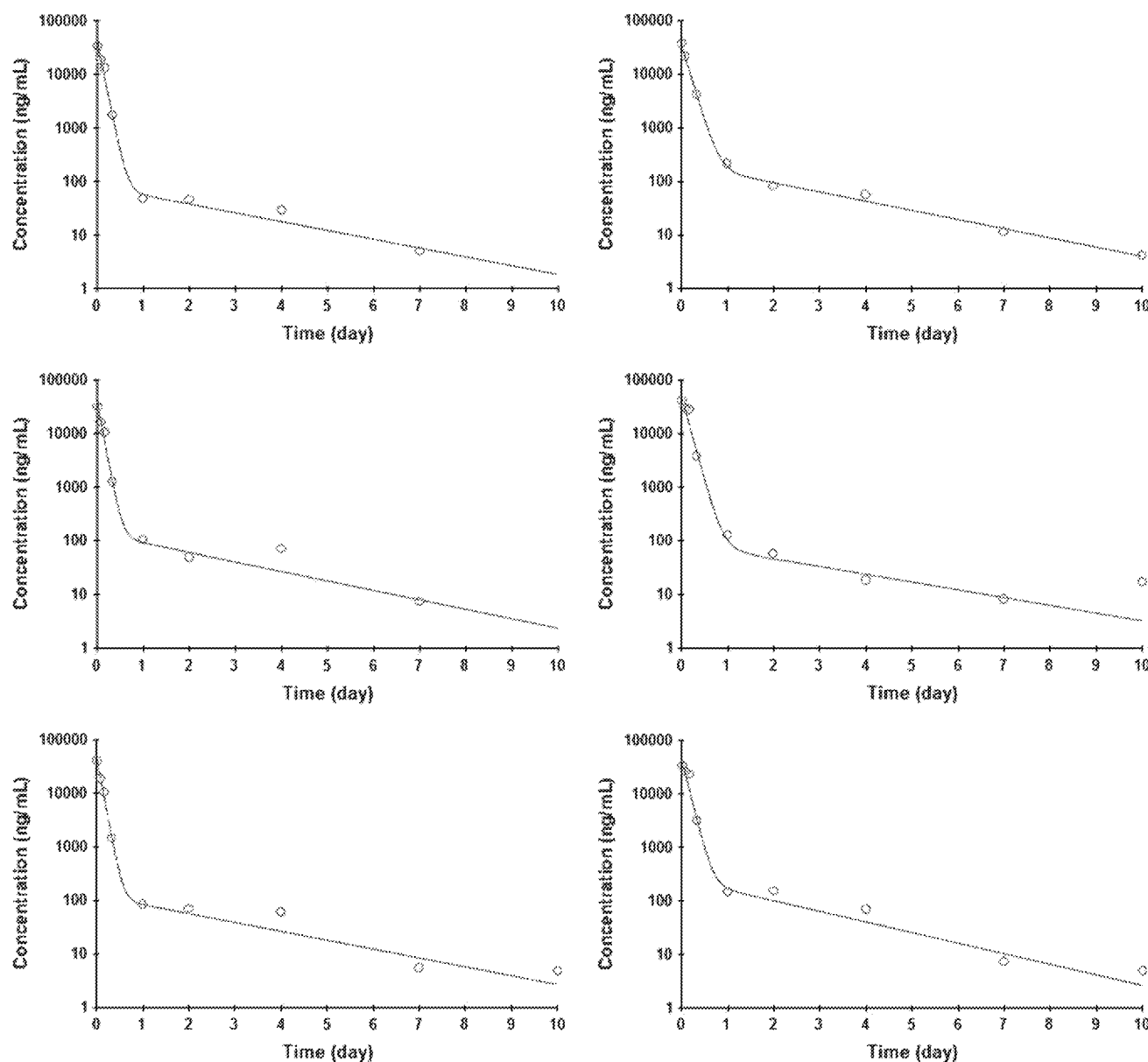[a]Total body clearance.

FIG. 5. Serum PFBA concentrations in male (left column) and female (right column) cynomolgus monkeys after a single iv dose of 10 mg K^+PFBA^-/kg body weight.

DISCUSSION

We believe that this is the first study of the pharmacokinetic characteristics of PFBA. The study is significant in that it covers multiple species and includes humans and potential exposure scenarios. The results of the pharmacokinetic investigations have been directly applied to the design of toxicological studies and to analysis of human biomonitoring data of people living in an area with PFBA present at low μg/l concentrations in drinking water. These studies have allowed insight on the potential for accumulation of body burden in humans on repeated exposure to PFBA-generating materials in the workplace and from exposure to environmental sources of PFBA. As such, the investigations will be of value in human-health-risk assessment.

From the rat and mouse data, it can be construed that absorption after an oral dose is rapid and relatively complete. After a single oral dose of 30 mg ammonium PFBA/kg, for male and female rats, respectively, mean K_a rates were 3 and 4/h, mean T_{max} times were 1.25 and 0.63 h, and mean C_{max} concentrations were 131 and 136 μg/ml (Table 2). In addition, after the same dose given iv, male and female rat C_{max} values (118 and 161 μg/ml) were similar to those obtained by giving the single oral dose (Table 2). The mean percent of dose in

TABLE 5

Serum PFBA Elimination Half-Life ($T_{0.5}$) with 95% CI for Exposed (to Materials that Metabolize to PFBA) 3M Company Cottage Grove, MN Production Site Volunteer Employees (subjects) during Removal from the Workplace by Study Subject for the Time Period from Initial Determination through End-of Study[a,b]

Subject	Serum PFBA elimination $T_{0.5}$ in h (95% CI)
1	105.3 (71.0–170.6)
2	109.7 (76.3–194.4)[c]
3	28.6 (N/A)[c,d,e]

[a] Excludes potential occupationally exposed time period.
[b] See also Figure 6.
[c] For subjects 2 and 3, calculations assumed the first value below the limit of quantitation (0.5 ng/ml) was equal to $0.5/2^{0.5}$.
[d] N/A = not applicable.
[e] There is no 95% CI calculated for subject #3 because a standard deviation of the elimination constant, λ, could not be calculated with only two data points.

TABLE 6

Initial and Final (Return-to-Work) PFBA Concentrations, Elapsed Time from Workplace, and Serum PFBA Elimination Half-Life ($T_{0.5}$, in h), by Subject Number, for 3M Company Cordova, IL Production Site Volunteer Employees

Subject (sex)	Initial [PFBA] (ng/ml)	Final [PFBA] (ng/ml)	Elapsed time (h)	Estimated $T_{0.5}$ (h)
1 (F[a])	2.0	< LLOQ[b]	234	118
2 (M[a])	12.1	0.6	228	53
3 (M)	5.7	1.0	180	72
4 (M)	12.5	0.8	180	44
5 (F)	4.7	< LLOQ	180	56
6 (M)	21.9	10.3	165	152
7 (M)	24.2	< LLOQ	261	47
8 (M)	10.6	1.5	180	63
9 (M)	71.0	4.9	180	47

[a] F = female employee, M = male employee.
[b] Value is less than the limit of quantitation (0.5 ng/ml). The limit of quantitation was used as the end value for purposes of estimating the serum PFBA elimination $T_{0.5}$.

male and female rat feces 24 h after a range of single oral doses of ammonium PFBA (3–300 mg/kg) ranged from 0.00% (based on concentrations in feces below the limit of quantitation) to 2.99% (Table 1). Absorption in mice was less complete than in rats; however, it was still efficient. Mice given single oral doses ranging from 10 to 100 mg/kg had mean T_{max} values ranging from 0.56 to 4.14 h (Table 3). Although C_{max} was similar for males and females at the 10 mg/kg dose, these values tended to differ between sexes and did not rise in linear proportion to dose through the dose range. The percent of dose recovered in feces 24 h after the dose ranged from 4.10 to 10.92% over the dose range. Even though it is not possible from these data to know the contribution of PFBA excreted in

bile to the fecal concentration, these data suggest relatively good absorption in rats and mice following single oral doses ranging up to 300 mg/kg.

Based on the values estimated for volume of distribution (V_{dss}) in this series of studies, PFBA appears to be distributed predominantly in extracellular space (V_{dss} approximating 200 ml/kg body weight). The V_{dss} values for male and female rats given 30 mg ammonium PFBA/kg single oral or iv doses ranged from 173 to 253 ml/kg with male values being somewhat higher than those of females and iv values being somewhat higher than oral values. For male and female mice, V_{dss} was estimated after single oral doses of 10, 30, and 100 mg ammonium PFBA/kg (Table 3). As in rats, male mice had
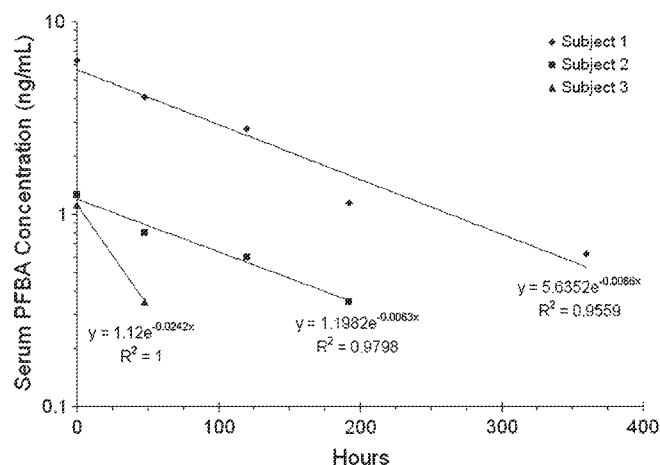

FIG. 6. Serum PFBA elimination curves for three 3M Company Cottage Grove production site employee volunteers. (Analysis was restricted to the time period which excluded any potential for occupational exposure.)

TABLE 7

Employee Status by Sex, Age, and Self-Reported Residential Water Source (Municipal or Private Well) among Participants ($N = 177$) Who had a Serum Sample for PFBA Analysis[a]

Employee status	Sex N (%)	Age Mean (95% CI)	Residential water source	
			Municipal N by sex (%)	Well N by Sex (%)
Former	M[b] = 106 (59.9)	65.8 (63.9–67.8)	M = 82 (61.7)	M = 24 (54.5)
	F[b] = 21 (11.9)	61.6 (57.0–66.2)	F = 15 (11.3)	F = 6 (13.6)
Current	M = 45 (25.4)	49.8 (47.8–51.8)	M = 32 (24.1)	M = 13 (29.5)
	F = 5 (2.8)	45.1 (32.4–57.9)	F = 4 (3.0)	F = 1 (2.3)
Total	177[c]		133	44

[a] Excludes two current employees with known occupational exposure.
[b] M = male, F = female.
[c] Total percents rounded to 100.

TABLE 8
Distribution of PFBA Serum Concentrations (ng/ml) by Employee Status and Self-Reported Residential Water Use[a]

[PFBA] (ng/ml)	Former employee (N = 127)			Current employee (N = 50)			All (former + current, N = 177)		
	Municipal N (%)	Well N (%)	Both N (%)	Municipal N (%)	Well N (%)	Both N (%)	Municipal N (%)	Well N (%)	Both N (%)
< LOQ (0.5)	70 (72.2)	23 (76.7)	93 (73.2)	23 (67.7)	11 (78.6)	34 (68.0)	93 (69.9)	34 (77.3)	127 (71.8)
0.5–< 1.0	15 (15.5)	2 (6.7)	17 (13.4)	11 (30.6)	2 (14.3)	13 (26.0)	26 (19.5)	4 (0.1)	30 (17.0)
1.0–< 2.0	7 (7.2)	4 (13.3)	11 (8.7)	1 (2.8)	1 (7.1)	2 (4.0)	8 (6.0)	5 (11.4)	13 (7.3)
2.0–< 3.0	3 (3.1)	0 (0.0)	3 (2.4)	1 (2.8)	0 (0.0)	1 (2.0)	4 (3.0)	0 (0.0)	4 (2.3)
3.0–< 4.0	1 (1.0)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.6)
4.0–< 5.0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
5.0–< 6.0	1 (1.0)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.6)
6.0–< 7.0	0 (0.0)	1 (3.3)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)	1 (0.6)
Total	97	30	127	36	14	50	133	44	177

[a]Excludes two current employees with known occupational exposure.

somewhat higher V_{dss} values than females. V_{dss} values for both males and females ranged from 107 to 296 ml/kg. In the monkey iv study, the V_{dss} was estimated to be approximately twice that of mice and rats. One possible explanation for the difference between the estimated rodent and monkey V_{dss} values is that the first blood sample obtained from the monkeys after the iv dose was at 30 min; whereas, for the rodents, samples were first obtained after 15 min. The rapid first-phase elimination in the monkeys may have skewed the estimate of the V_{dss}.

Although an analysis of tissue uptake was not undertaken in this series of studies, liver PFBA concentration data were obtained in rats and mice. These data can be compared with serum PFBA concentrations. Mean liver concentrations in male rats (liver concentration data were not obtained for female rats due to the very low concentrations encountered) were approximately 22–27% of respective serum concentrations over the dose range of 3–100 mg ammonium PFBA/kg (data from Table 1). In male mice, mean liver PFBA concentrations as a percent of serum PFBA concentrations ranged from 22 to 28% over the dose range of 10–100 mg/kg; however, in females, this measure remained fairly constant at 17% across the dose range (data from Table 3). This ratio suggests that the majority of the PFBA in liver was contributed by the blood (serum) present in the liver (Castagna, 1965), as livers were not perfused before samples were taken for analysis. The lower concentrations in liver tissue relative to serum concentration that were observed in rats and mice suggest that uptake of PFBA by the liver is limited. Additional studies will be needed to determine the bioavailability of blood-borne PFBA to other tissues.

These studies have demonstrated that PFBA is efficiently eliminated in several species (mice, rats, monkeys, and humans) with elimination rates resulting in serum PFBA elimination $T_{0.5}$ values of a few h for rats and mice to approximately 2–4 days for monkeys (mean 43 h after iv dose)

and humans (48–96 h as determined in two separate studies). Unlike the data for the higher chain homologue PFOA (Andersen *et al.*, 2006; Hundley *et al.*, 2006; Kudo and Kawashima, 2003; Olsen *et al.*, 2007a), the present study data do not indicate substantial differences exist in the serum elimination of PFBA across species, including humans, as half-lives ranged between several h to a few days. Comparing sex-specific elimination rates in rats with those of mice at the 30 mg/kg oral dose, the mean serum PFBA elimination half-life in rats was 57% of the mean elimination rate in mice for both sexes.

Sex differences in elimination rate were evident between males and females for mice and rats, but not for humans and cynomolgus monkeys. In most cases with mice and rats, male elimination rate was about one-fifth that of females. In male mice, the elimination rate increased by a factor of
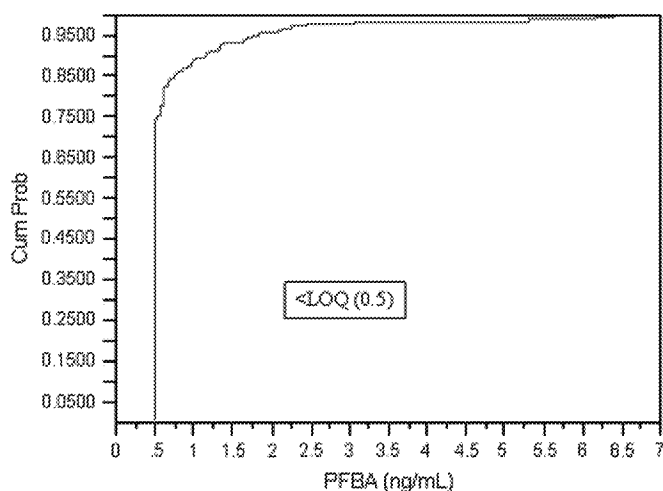

FIG. 7. Cumulative probabilities of serum PFBA concentrations among 177 individuals with potential exposure to PFBA through drinking water.

approximately three at the 100 mg/kg dose when compared with the rate at the two lower doses (10 and 30 mg/kg), and the elimination rate was roughly half that of females in 100 mg/kg dose group. The faster $T_{0.5}$ for male mice at the 100 mg/kg dose suggests that the simple one-compartment model is not adequate to describe the kinetic data at 100 mg/kg and that a two-compartment model may be more appropriate. The faster elimination rate in female rats is similar to but much less pronounced than that which has been observed for the higher chain homologue, PFOA (Hundley et al., 2006; Kennedy et al., 2004; Kudo and Kawashima, 2003). In contrast to mice and rats, sex differences in elimination rates were not evident in monkeys and humans. The elimination rate obtained from the two female employees from the 3M Cordova, IL facility were similar to the data obtained for male employees, indicating a lack of sex difference in human elimination of PFBA and consistent with the lack of a sex difference in serum PFBA elimination between the three male and three female cynomolgus monkeys.

Human, monkey, and male rat serum PFBA elimination rates are markedly faster (much shorter half-lives) than the serum elimination rates observed for the eight-carbon homolog, PFOA, in these species (Butenhoff et al., 2004; Kudo and Kawashima, 2003; Olsen et al., 2007a). For humans, a geometric mean serum elimination $T_{0.5}$ of 1273 days (95% CI 1083–1495) was determined in a 5-year follow-up of retired fluorochemical production workers (Olsen et al., 2007a). Mean serum elimination $T_{0.5}$ for PFOA in monkeys is approximately 20–30 days. For male rats, serum PFOA elimination half-life is several days. Recent data have demonstrated a role for a renal tubular reabsorption transporter (oatp1) in the sex difference observed in PFOA elimination in rats (Katakura et al., 2007), and the cynomolgus monkey pharmacokinetic data for PFOA fit a model that incorporates the concept of renal tubular reabsorption (Andersen et al., 2006). Therefore, it is possible that differences in elimination of perfluoroalkyl carboxylates based on chain length, sex, and species may be largely influenced by specific differences in reabsorption of these compounds via renal tubular reabsorption transporters.

In the laboratory animal species studied, urine was the primary route of elimination. Male rats and mice excreted approximately one-half the amount in urine that their respective females excreted over a 24-h period following a single oral dose over a range of doses. One exception was that, for male rats given a 300 mg/kg dose of ammonium PFBA, the percent of dose as PFBA eliminated in urine over a 24-h period was 90% as compared with 51–62% at doses between 10 and 100 mg/kg. The percent of dose excreted in urine over 24 h corresponds to the relative differences in elimination rate. The presence of higher percentages of dose in feces for male and female mice (range of means from 4.10 to 10.92%) than in rats (range of means from 0.00 to 2.99%) suggests less complete absorption, biliary elimination, or, more likely, some combination of both. In male and female cynomolgus

monkeys, the percent of dose excreted in 24 h was similar to that of male mice.

Renal tubular reabsorption may also factor into PFBA pharmacokinetics. The observation that male rats given 300 mg/kg ammonium PFBA eliminated significantly more PFBA in urine over a 24-h period postdose than male rats given lower doses (vida supra) suggests saturation of a putative renal tubular reabsorption process for PFBA, as described for PFOA (Andersen et al., 2006; Katakura et al., 2007). In addition, a two-compartment model better described data for the monkeys, as a second elimination phase became apparent when serum PFBA concentrations had decreased to between approximately 10 and 100 ng/ml. There was also a suggestion of this in the data from the female mice, with a second phase possibly present beginning at serum PFBA concentrations in the same range (10–100 ng/ml); although, additional work would be needed to confirm this. Although a one-compartment model adequately described the data from male mice given 10 and 30 mg/kg ammonium PFBA, the shorter half-life in male mice given 100 mg/kg ammonium PFBA compared with those given 10 and 30 mg/kg also suggests that a two-compartment model may be more appropriate for the data from male mice at the 100 mg/kg dose. The second compartment could represent more tightly sequestered PFBA or, possibly, a renal tubular reabsorption process that is saturable at higher serum concentrations, as describe above.

For humans, the average serum PFBA elimination $T_{0.5}$ in adult males and females likely is between 2 and 4 days, based on data obtained for 12 individuals. The data need to be interpreted cautiously, because the workers were not exposed directly to PFBA and were exposed to materials that, given their chemical structures, likely are metabolized to PFBA via oxidation or hydrolysis (Jay F. Schulz, 3M Company, personal communication). The presence of PFBA in the blood of workers exposed to these compounds provides evidence of this. However, it was not known to what extent and at what rate these individual compounds would contribute to the PFBA measured in the serum. It was recognized that different rates of metabolism to PFBA for multiple compounds could confound an estimation of the true elimination half-life of PFBA from the serum of these individuals. On the other hand, it was also recognized that multiple postexposure serum and urine collections over a number of days would provide a reasonably accurate estimate of human PFBA elimination despite the uncertainty as to the specific origins of the measured PFBA in the serum of these workers.

It should be expected that a population exposed to PFBA in its drinking water at concentrations of 1 to 2 µg/l PFBA, as reported by the Minnesota Department of Health (MDH, 19 January 2007 newsletter), would not result in an accumulation of PFBA in their sera above a few ng/ml. To test this hypothesis, assume the following factors for an adult individual: (1) a water source containing 2 µg/l PFBA, as has been reported by the MDH for the higher concentrations

measured in municipal water in communities of southeast Minneapolis–St Paul metropolitan area; (2) a 70 kg adult drinks 2 l of municipal and/or well water per day; (3) the V_{dss} of PFBA is 200 ml/kg; and (4) humans have a serum PFBA elimination $T_{0.5}$ equal to 4 days. Based on these assumptions, the amount of PFBA consumed daily would be 4 μg. Based on a first-order elimination model, at steady state these assumptions would yield a serum PFBA concentration of approximately 0.0016 μg/ml (1.6 ng/ml). In our biomonitoring study, reported herein, 96% of the 177 former and current 3M employees of the Cottage Grove site had serum PFBA concentrations < 2 ng/ml. Therefore, the human serum PFBA elimination rate estimated by our study is consistent with the observations from the biomonitoring study reported herein and the drinking water PFBA concentrations reported by MDH.

There are several factors that need to be considered in the evaluation of the present community study results. The study population was not a representative sample of the affected communities. Few individuals resided in those communities where water concentrations have been reported to be higher (in the 1–2 μg/l range) for PFBA than in other local communities. Samples represented many individuals who were older than the average age in these counties. Children were not part of the sample size. Blood samples were collected in 2005 not in 2007; although, it is likely that the PFBA was present for some time in the ground water.

In summary, the pharmacokinetic profile of PFBA was evaluated for multiple species, including mice, rats, monkeys, and humans. We believe this to be the first evaluation of the pharmacokinetics of PFBA. Our findings demonstrate that PFBA is eliminated efficiently from serum with a low potential for accumulation from repeated exposure.

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